Low-phytate barley cultivars improve the utilization of phosphorus, calcium, nitrogen, energy, and dry matter in diets fed to young swine

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Low-phytate barley cultivars improve the utilization of phosphorus, calcium, nitrogen, energy, and dry matter in diets fed to young swine[1]

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ABSTRACT: A 28-d experiment was conducted using 45 crossbred barrows with an average initial BW of 9.5 kg and age of 35 d to evaluate low-phytate barley (LPB) mutants (M) M422, M635, and M955, which were hulled, near-isogenic progeny of the normal barley (NB) Harrington and had 47, 66, and 80% less phytic acid, respectively, than NB. A hull-less LPB, M422-H, which was not near-isogenic to the other cultivars, was also evaluated. Apparent nutrient balance, bone measurements, and growth performance were the response criteria evaluated. The barrows were fed the diets to appetite in meal form in individual metabolism crates. Barley and soybean meal were the only sources of phytic acid. Dietary protein supplementation and ME/kg were equalized in all diets. The treatments were diets containing NB, M422, M635, or M422-H without or with added inorganic P (iP), or M955 without added iP. Diets with added iP contained 0.30% available P (aP), the same concentration of aP provided by the diet containing M955 without added iP. There were linear increases ($P \leq 0.02$) in ADG, G:F, metacarpal and radius bone strength, and fat-free dry weight, and in the absorption and retention (g/d and % of intake) of P and Ca with increasing dietary concentration of aP from the near-isogenic cultivars NB, M422, M635, or M955 without added iP. There were linear decreases in the grams ($P \leq 0.02$) and percentages ($P < 0.001$) of P and Ca excreted per day with increasing dietary concentration of aP without added iP. There were no responses for N or energy balance. Growth performance and bone response criteria did not differ for barrows fed the diet containing M955 or the near-isogenic diets containing NB, M422, or M635 with added iP. However, barrows fed the diet containing M955 had greater ($P \leq 0.02$) percentages of P, N, and energy absorption and retention, Ca absorption, and DM digestibility and had less ($P \leq 0.02$, g/d and %) excretion of P, N, energy, and Ca (g) per day than barrows fed the diets containing the near-isogenic NB or LPB cultivars with added iP. When dietary aP was equalized with iP, the excretion of P in feces plus urine (g/d) was reduced by 20.2, 27.9, and 44.6%, respectively, in barrows fed the diets containing M422 + iP, M635 + iP, or M955 compared with barrows fed the diet containing NB + iP. Energy utilization did not differ for barrows fed the diets containing hulled or hull-less LPB when ME/kg was equalized with lard. In conclusion, the apparent utilization of P and Ca, the bone strength and fat-free dry weight, and growth performance increased with increasing dietary concentration of aP provided by LPB, in association with linear decreases in P and Ca excretion. Barrows fed the diet containing M955 also had greater utilization and less excretion of P, Ca, N, energy, and DM than barrows fed the diets containing the near-isogenic NB or LPB cultivars with added iP to equalize aP at 0.30%.

Key words: barley, nutrient absorption, nutrient excretion, phytate, pig, swine

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INTRODUCTION

About 70% of the P in barley is bound in the form of phytic acid or phytate (myo-inositol hexakis-dihydrogen phosphate; Maga, 1982; Reddy et al., 1989; Lott et al., 2000). The enzyme phytase is required for phytate digestion in the small intestine, and swine produce little to no intestinal phytase (Pointillart et al., 1984, 1987; Crenshaw, 2001). Although some microbial hydrolysis of phytate does occur in the hindgut of the pig (Leytem et al., 2004; Angel et al., 2005), and variable amounts of P and Ca are absorbed from the cecum and colon (Partridge, 1978; Liu et al., 2000), swine excrete most of the phytate P in their manure (Cromwell and Coffey, 1991; Veum et al., 2001, 2002). However, P absorption may be increased and P excretion reduced in swine by

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supplementation of low-P plant ingredient-based diets with an effective phytase enzyme product (Harper et al., 1997; Liu et al., 1998; Veum et al., 2006).

The development of low-phytic acid grains (Larson et al., 1998; Raboy et al., 2001; Dorsch et al., 2003) and soybeans (Wilcox et al., 2000; Hitz et al., 2002; Oltmans et al., 2005) has the potential to increase nutrient absorption and reduce nutrient excretion of minerals such as P and Ca among nonruminant animals and humans globally. Barley is climate tolerant and may be grown from the subarctic to the subtropical regions, and it ranks fourth in grain production globally (US Grains Council, 2006).

The objectives of this experiment were to evaluate 3 hulled, mutant, low-phytic acid barley (**LPB**) cultivars that have decreased concentrations of phytic acid and are near-isogenic progeny of a hulled, normal barley (**NB**) cultivar, and to evaluate a hull-less LPB compared with the corresponding hulled LPB. Apparent nutrient absorption, nutrient excretion, bone breaking strength, and growth performance were the response criteria evaluated.

MATERIALS AND METHODS

Animals and Housing

This experiment was approved by the University of Missouri Animal Care and Use Committee.

Forty-five crossbred, Yorkshire-Landrace-Duroc barrows with an average BW of 9.5 ± 0.3 kg and age of 35 ± 1 d were allotted to 9 treatments in a completely random design in a 28-d experiment. The barrows were placed in individual, elevated, solid-walled, stainless-steel metabolism crates (floor space: 1 replication with 0.7 m^2, 3 replications with 0.9 m^2, and 1 replication with 1.3 m^2/pig) equipped with stainless-steel nipple drinkers, feeders, and woven wire or slotted flooring, with 5 replications per treatment. The room temperature was maintained at 26°C (range of 25.4 to 26.6°C) during wk 1 with thermostatically controlled heaters and exhaust fans, and lowered by 1°C each week thereafter, with 15 h of light beginning at 0600. The barrows were fed the air-dried diets in meal form to appetite twice daily (0730 and 1600).

Barley Cultivars and Dietary Treatments

The barley cultivars studied in this experiment were produced at the National Small Grains Germplasm Research Facility (Aberdeen, ID) and transported to the University of Missouri. The NB was the hulled cultivar Harrington that was homozygous for the "wild-type" alleles of the phytic acid genes, and produced grain with normal concentrations of phytic acid. The mutant (**M**) LPB cultivars M422, M635, and M955 were isolated in the cultivar Harrington (Dorsch et al., 2003) and are near-isogenic variants of Harrington that produced grains with approximately 47, 66, and 80% less phytic acid, respectively, than NB (Table 1). The hulled barley M422 is homozygous for the *low phytic acid 1-1* allele of the barley *low phytic acid 1* gene, whereas M635 is homozygous for the *low phytic acid 3-1* allele of the barley *low phytic acid 3* gene (Larson et al., 1998; Roslinsky et al., 2007). The gene and allele identity of the hulled M955 has not been determined. A hull-less LPB was produced by crossing M422 with a hull-less line derived from the cultivar Phoenix, producing hull-less M422 (**M422-H**). Because of the crossing required to remove the hulls, M422-H is not near-isogenic with the hulled LPB and NB cultivars.

The barley cultivars and soybean meal (**SBM**) were the only sources of phytic acid in the diets (Table 2). Diets 1 to 4 contained the near-isogenic cultivars NB (control), M422, M635, and M955, respectively, in order of increasing concentration of available P, without added inorganic P (**iP**). Available P (**aP**) was calculated by subtracting the analyzed concentration of phytic acid P from the analyzed concentration of total P (**tP**). Diet 4 contained M955 and provided 0.30% aP without supplemental iP. Therefore, diet 4 supplied adequate aP for the barrows during wk 2 to 4 of this experiment. Diets 5 to 7 contained NB, M422, or M635, respectively, with added iP from monosodium phosphate (diets **NB + iP**, **M422 + iP**, and **M635 + iP**, respectively) to equal the aP concentration in the diet containing M955. Diet 8 contained M422-H without added iP, and diet 9 contained M422-H with added iP (**M422-H + iP**; 0.30% aP).

Protein ingredient supplementation was equalized across all dietary treatments to standardize the biological value and the addition of phytate from SBM. Therefore, dietary CP ranged from 19.11 to 20.52% among treatments (Table 2). Crystalline AA supplementation was used to meet the essential AA requirements. Dietary Ca was equalized at approximately 0.6% across all treatments, 0.1% below the NRC (1998) requirements at the beginning of the experiment. Dietary Ca was lowered in the experimental diets to avoid the negative effects of a wide range of Ca:P ratios on pig growth performance (Lei et al., 1994; Cromwell et al., 1995; Qian et al., 1996), bone development (Nielsen et al., 1971), and the digestibility of P and other nutrients (Liu et al., 1998; Johnston et al., 2004). Therefore, the Ca:tP ratios were 1.2:1 and 1.5:1 for the diets with or without iP supplementation, respectively. Metabolizable energy was equalized at 3.34 Mcal/kg (calculated basis) by increasing lard (at the expense of barley) from 1.51% in the diets with hull-less barley (diets 8 and 9) to 6.30% in the diets with hulled barley (diets 1 through 7).

Measurements

Barley Cultivar and Diet Analysis. Before diet formulation, stocks of the barley cultivars and SBM (Table 1) and the spray-dried whey and spray-dried blood cells used in the experimental diets were sampled and analyzed in triplicate for proximate analysis components

Table 1. Analyzed chemical composition (%) of air-dried barley cultivars and soybean meal, as-fed basis[1]

	Barley cultivars					
Item	NB, wild-type control	M422	M635	M955	Hull-less M422	Soybean meal
DM	88.1	88.0	89.2	88.2	88.7	87.9
Ash	2.30	2.13	2.14	2.33	1.50	6.45
Crude fat	1.72	2.11	1.46	1.32	2.36	1.28
Crude fiber	3.17	3.98	3.58	3.74	1.10	3.51
CP	9.56	10.19	11.12	11.26	11.43	47.76
Lys	0.38	0.39	0.41	0.42	0.44	3.01
Met	0.18	0.18	0.19	0.20	0.20	0.71
Cys	0.24	0.25	0.27	0.27	0.27	0.81
Trp	0.10	0.10	0.11	0.12	0.11	0.66
Thr	0.32	0.34	0.36	0.37	0.37	1.84
Ile	0.33	0.34	0.38	0.37	0.39	2.15
Val	0.47	0.48	0.53	0.52	0.56	2.25
Ca	0.06	0.06	0.06	0.06	0.06	0.34
Total P	0.36	0.32	0.35	0.35	0.34	0.70
Phytic acid P	0.24	0.11	0.08	0.05	0.12	0.40
Available P[2]	0.12	0.21	0.27	0.30	0.22	0.30
GE, Mcal/kg	4.07	4.06	4.04	4.00	4.08	4.35

[1]Average of triplicate samples. NB = normal barley; M = mutant.
[2]Calculated by subtracting phytic acid P from total P.

(AOAC, 1990), GE by oxygen bomb calorimetry (Parr Instrument Co., Moline, IL), and complete AA (Benson and Patterson, 1971). The samples were hydrolyzed under N with 6 *N* HCl for 24 h at 110°C before AA analysis was performed by automated cation-exchange chromatography. Analysis for cystine and methionine involved performic acid oxidation before hydrolysis. Tryptophan was determined by the method of Spies and Chambers (1949). Triplicate samples of the above ingredients plus monosodium phosphate and ground limestone were digested using a wet ash procedure (AOAC, 1990). The digests were analyzed for the concentrations of tP by the molybdovanadate colorimetric method (Spectra Rainbow Microplate Reader, Tecan, Inc., Durham, NC) and for the concentrations of Ca by atomic absorption spectrophotometry (Spector AA-30, Varian Analytical Instruments, San Fernando, CA). Subsamples of the barley cultivars and SBM were analyzed in triplicate for phytic acid P, as described by Raboy et al. (2000). Subsamples of the barley cultivars were analyzed in sextuplet for endogenous phytase activity, as described by Zyla et al. (2002). Endogenous phytase activity averaged 285 ± 15 units/kg for all of the barley cultivars, with no relationship between endogenous phytase activity and aP (or phytic acid) concentration of the barley cultivars. The analyzed nutrient values of the ingredients (calculated values for ME) were used to formulate the experimental diets. After the diets were mixed, all of the diets were sampled and analyzed in triplicate, as previously described, for P, Ca, N, GE, and DM, and for Cr_2O_3 (AOAC, 1990) by atomic absorption spectrophotometry.

Animal and Bone Measurements. Feed consumption of individual barrows was determined weekly and for d 22 to 26 for apparent nutrient balance. The barrows were weighed at the beginning and end (d 0 and 28) of the test. On d 28, the barrows were killed (stunned by captive bolt followed by exsanguination). The right front foot and elbow of each pig was removed and stored at 2°C in a plastic bag. The third metacarpal and radius bones were excised and cleaned of all adhering tissue within 3 d for bone size and weight measurements, and for the determination of breaking strength and ash weight. A caliper (Model CDS6, Mitutoyo Corp., Kawasaki, Japan) was used to measure the metacarpal and radius bone lengths and the midshaft widths at the narrowest and widest points. Breaking strength of the fresh bones was determined using an Instron testing machine (Model TML, Instron Corp., Canton, MA), similar to the procedure described by Crenshaw (1986). Force was applied to the center of the bone, which was held by 2 supports spaced 3.0 cm apart. After determination of the breaking strength, the bones were wrapped with cheesecloth, boiled in deionized water for 2 h, dried at 55°C for 24 h, and extracted with ethyl ether for 4 d. Ash weight was determined after the fat-free bones were dried at 55°C and 100°C for 18 and 2 h, respectively, and ashed in a muffle furnace at 600°C for 16 h (AOAC, 1990).

Apparent Nutrient Balance. Diets contained 0.05% Cr_2O_3 at the expense of barley as an indigestible indicator. Fecal samples (about 100 g of DM without feed contamination) and total urine collections were made twice daily from d 22 to 26. Fecal samples were stored in plastic freezer bags. Urine was collected in plastic pails containing 30 mL of 6 *N* HCl. The total urine volume was recorded, and 10% was saved in 1-L, wide-mouthed, screw-capped plastic bottles. Fecal and urine

Table 2. Ingredient and chemical composition (%) of air-dried basal diets, as-fed basis[1]

	Diet number and description								
	1	2	3	4	5	6	7	8	9
Item	NB, wild-type control	M422	M635	M955	NB + iP	M422 + iP	M635 + iP	Hull-less M422	Hull-less M422 + iP
Ingredient									
Barley cultivar	53.53	53.54	53.54	53.54	53.13	53.31	53.46	58.42	58.28
Soybean meal (48% CP)	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00
Spray-dried whey	7.75	7.75	7.75	7.75	7.75	7.75	7.75	7.75	7.75
Spray-dried blood cells	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Lard	6.30	6.30	6.30	6.30	6.30	6.30	6.30	1.51	1.51
Lactose	5.25	5.25	5.25	5.25	5.25	5.25	5.25	5.25	5.25
Ground limestone	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.84	0.84
Salt, noniodized	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Trace mineral[3]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Lys HCl	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.05	0.05
DL-Met	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01
L-Thr	0.03	0.02	0.02	0.02	0.03	0.02	0.02	—	—
Tylan[4]	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Monosodium phosphate[5]	—	—	—	—	0.40	0.23	0.08	—	0.14
Chromic oxide	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Chemical composition, analyzed									
Ca	0.61	0.60	0.60	0.61	0.61	0.61	0.60	0.61	0.62
Total P	0.41	0.39	0.40	0.41	0.50	0.50	0.47	0.42	0.53
Available P[6]	0.20	0.24	0.28	0.30	0.30	0.30	0.30	0.26	0.30
CP	19.39	19.79	19.96	20.24	19.14	19.54	19.91	20.32	19.11
Lys	1.23	1.23	1.24	1.24	1.23	1.23	1.23	1.24	1.24
Met + Cys	0.66	0.66	0.68	0.68	0.66	0.66	0.68	0.69	0.69
Trp	0.24	0.24	0.25	0.25	0.24	0.24	0.25	0.25	0.25
Thr	0.75	0.75	0.75	0.77	0.75	0.75	0.76	0.77	0.77
Ile	0.73	0.74	0.76	0.75	0.73	0.74	0.76	0.78	0.78
GE, Mcal/kg	4.20	4.24	4.26	4.28	4.18	4.22	4.24	4.05	4.01
ME,[7] Mcal/kg	3.34	3.34	3.34	3.34	3.33	3.34	3.34	3.34	3.34

[1]NB = normal barley; M = mutant; iP = inorganic P.
[2]Vitamin premix provided, per kilogram of diet: 11,000 IU of vitamin A as retinyl acetate; 1,100 IU of vitamin D_3; 4.4 IU of vitamin E from DL-α-tocopheryl acetate; 4.0 mg of vitamin K from menadione sodium dimethylprimidinol bisulfite; 30.3 μg of vitamin B_{12}; 8.3 mg of riboflavin; 28.1 mg of pantothenic acid as D-calcium pantothenate; 33.1 mg of niacin; 551.3 mg of choline from choline chloride; 220.5 μg of biotin from D-biotin; and 1.65 mg of folic acid.
[3]Trace mineral premix provided per kilogram of diet: 110 mg of Zn as $ZnSO_4$; 110 mg of Fe as $FeSO_4$; 22 mg of Mn as $MnSO_4$; 11 mg of Cu as $CuSO_4$; 0.2 mg of I as $Ca(I0_3)_2$; and 0.2 mg of Se as Na_2SeO_3.
[4]Tylan provided 110 mg of tylosin/kg of diet (Elanco Global, Ely Lilly & Co., Greenfield, IN).
[5]Monosodium phosphate contained 25.3% P.
[6]Calculated by subtracting phytic acid P from total P.
[7]Calculated by using NRC (1998) values.

samples were immediately frozen at –20°C until analyzed. Each pig crate, fecal collection screen, and urine collection pail was washed immediately after collection.

The 5-d fecal collections for individual barrows were thawed, pooled, mixed, and dried in an oven at 55°C for 48 h. The dried fecal samples and samples of each diet were ground to pass a 1-mm screen (Wiley mill, model No. 4, Arthur H. Thomas Co., Philadelphia, PA). The 5-d urine collections for individual barrows also were thawed, mixed, and subsampled for analysis. The feed, fecal, and urine samples were analyzed in triplicate using the analytical procedures previously described for analyzing the barley cultivars. The analyzed diet values were used to determine the apparent balance of P, Ca, N, and energy, and the digestibility of DM.

Statistical Analysis

All data were analyzed by ANOVA as a randomized complete block design (Snedecor and Cochran, 1989) using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Individual barrows were the experimental units. The preplanned, single df comparisons were the linear and quadratic responses for diets 1 to 4 (NB, M422, M635, and M955, respectively), and the comparisons of diet 4 vs. diets 5 + 6 + 7 (M955 vs. the near-isogenic diets NB + iP, M422 + iP, and M635 + iP, respectively, all with 0.30% aP), diet 2 vs. diet 8 (M422 vs. M422-H), and diet 9 (M422-H + iP) vs. diets 4 + 5 + 6 + 7, all with 0.30% aP. Significance was reported at $P \leq 0.05$, with a trend at $P > 0.05$ and $P \leq 0.10$.

Table 3. Effect of mutant barley cultivars on growth performance and bone characteristics of barrows[1]

	Diet number and description									
	1	2	3	4	5	6	7	8	9	
Item	NB, wild-type control	M422	M635	M955	NB + iP	M422 + iP	M635 + iP	Hull-less M422	Hull-less M422 + iP	SEM
Pig BW, kg										
d 0	9.41	9.47	9.48	9.47	9.48	9.54	9.63	9.50	9.56	0.34
d 28	22.19	23.94	25.04	25.12	27.05	27.80	25.90	23.18	25.54	0.95
ADG, g	456	517	556	559	628	652	581	489	535	27
ADFI,[2] g	838	873	908	906	971	1001	933	902	929	40
G:F, g/kg	546	591	614	617	648	651	624	541	576	18
Metacarpal bone										
Breaking strength, kg	24.98	28.54	28.08	33.74	40.14	37.40	32.18	30.00	35.40	2.40
Fresh wt, g	8.71	9.47	9.93	9.95	10.32	10.41	9.51	9.66	9.60	0.53
Fat-free dry wt, g	2.27	2.55	2.79	2.90	3.12	3.08	2.76	2.79	2.81	0.15
Ash wt, g	1.05	1.20	1.34	1.41	1.58	1.45	1.28	1.19	1.42	0.14
Bone length, mm	50.20	51.11	53.27	52.25	53.04	52.06	51.78	51.03	50.79	0.90
Radius bone										
Breaking strength, kg	43.14	41.92	50.66	65.02	69.26	66.14	45.46	54.54	67.90	5.92
Fresh wt, g	25.30	27.31	27.49	28.40	30.04	29.55	28.39	27.93	27.71	1.39
Fat-free dry wt, g	7.11	7.65	8.13	8.83	9.78	9.46	8.75	8.09	8.87	0.45
Ash wt, g	3.37	3.65	4.02	4.53	5.13	4.93	4.47	3.92	4.60	0.27
Bone length, mm	77.58	79.60	80.90	81.45	79.83	80.94	80.35	79.61	78.94	1.29

[1]NB = normal barley; M = mutant; iP = inorganic P.
[2]As-fed basis.

RESULTS

Growth Performance and Bone Characteristics

There were linear increases ($P \leq 0.02$) in ADG, G:F, and the breaking strength and fat-free dry weight of the metacarpal and radius bones with increasing dietary concentrations of aP in the diets containing NB, M422, M635, or M955, respectively, all without added iP (Tables 3 and 4). In addition, there were linear increases in the metacarpal ($P \leq 0.08$) and radius ($P \leq 0.05$) bone ash weight and length with increasing dietary concentrations of aP from these cultivars without added iP. There were no differences in the growth performance or bone measurement response criteria for barrows fed the diet containing M955 with 0.30% aP and barrows fed the diets containing the near-isogenic barley cultivars NB, M422, or M635 with added iP to provide 0.30% aP. For the diets containing M422 or M422-H (diet 2 vs. diet 8, respectively, both without added iP), barrows fed the diet containing M422 tended to have a greater ($P = 0.06$) G:F than barrows fed the diet containing M422-H. Barrows fed the diet containing M422-H + iP (diet 9) had a lower ($P \leq 0.03$) ADG and G:F than barrows fed the other barley diets containing 0.30% aP (diets 4 + 5 + 6 + 7), whereas barrows fed these diets were not different in metacarpal or radius bone strength or other bone measurements. There were no treatment comparison differences for the metacarpal or radius bone midshaft widths taken at the narrowest (11.4 ± 0.4 and 10.4 ± 0.4, respectively) or widest (13.6 ± 0.4 and 16.3 ± 0.6, respectively) points (data not provided).

Apparent P, Ca, and N, and Energy Balance and DM digestibility

There were linear increases in the apparent absorption and retention of P ($P < 0.001$) and Ca ($P \leq 0.02$) in grams per day and as percentages of intake with increasing dietary concentrations of aP in the diets containing NB, M422, M635, or M955, respectively, without added iP (Tables 5 and 6). In addition, there were linear decreases in the grams of fecal P ($P < 0.001$) and Ca ($P \leq 0.02$) excreted per day, and in the percentages ($P < 0.001$) of P and Ca excreted (feces plus urine) with increasing dietary concentrations of aP from these cultivars without added iP. There were no linear responses for N and energy balance (amounts/d or as % of intake) or the percentage of DM digestibility, although there were quadratic responses ($P \leq 0.10$) for grams of fecal N and kilocalories of urinary energy excreted daily.

Barrows fed the diet containing M955 consumed and excreted fewer ($P < 0.001$) grams of P per day, fewer ($P \leq 0.02$) grams of Ca and N per day, and fewer ($P = 0.01$) kilocalories of energy per day than barrows fed the diets containing the near isogenic cultivars NB, M422, or M635 with added iP to provide 0.30% aP. Expressed as a percentage of intake, the absorption and retention of P, N, and energy; the absorption of Ca; and DM digestibility were greater ($P \leq 0.02$) for barrows fed the diet containing M955 than for barrows fed the diets

Table 4. Statistical significance of growth performance and bone characteristics of barrows[1]

	P-value				
Item	Linear	Quadratic	Diet 4 vs. diets 5 + 6 + 7	Diet 2 vs. diet 8	Diet 9 vs. diets 4 + 5 + 6 + 7
Pig BW, kg					
d 0	0.960	0.960	0.826	0.950	0.937
d 28	0.042	0.203	0.522	0.575	0.078
ADG, g	0.012	0.122	0.475	0.460	0.025
ADFI,[2] g	0.236	0.463	0.413	0.622	0.607
G:F, g/kg	0.010	0.101	0.938	0.060	0.005
Metacarpal bone					
Breaking strength, kg	0.019	0.800	0.440	0.670	0.863
Fresh wt, g	0.120	0.312	0.816	0.801	0.445
Fat-free dry wt, g	0.006	0.250	0.991	0.242	0.356
Ash wt, g	0.073	0.541	0.947	0.942	0.921
Bone length, mm	0.080	0.092	0.716	0.953	0.149
Radius bone					
Breaking strength, kg	0.005	0.531	0.548	0.141	0.338
Fresh wt, g	0.166	0.600	0.848	0.752	0.378
Fat-free dry wt, g	0.009	0.754	0.569	0.499	0.517
Ash wt, g	0.003	0.860	0.504	0.476	0.582
Bone length, mm	0.045	0.340	0.250	0.996	0.247

[1]The linear and quadratic contrasts included diets 1 to 4 that contained normal barley, Mutant 422, Mutant 635, or Mutant 955, respectively, without added inorganic P (iP). Diet 4 provided 0.30% available P (aP) without added iP. Diets 5, 6, and 7 contained normal barley, Mutant 422, or Mutant 635, respectively, with added iP to provide 0.30% aP. Diet 8 contained hull-less Mutant 422 without added iP. Diet 9 contained hull-less Mutant 422 with added iP to provide 0.30% aP.
[2]As-fed basis.

containing NB, M422, or M635 with added iP to provide 0.30% aP. In addition, the percentages of P, N, and energy excreted (fecal + urinary) were less ($P \leq 0.02$) for barrows fed the diet containing M955 than for barrows fed the diets containing NB, M422, or M635 with added iP to provide 0.30% aP.

Barrows fed the diet containing M422 tended to retain more ($P = 0.07$) grams of N per day and excrete fewer grams of fecal P ($P = 0.10$) and urinary N ($P < 0.001$) per day than barrows fed the diet containing M422-H (diet 2 vs. diet 8, respectively). Expressed as a percentage of intake, P absorption and retention were greater ($P \leq 0.06$) and P excretion (fecal + urinary) was less, and N retention was greater ($P < 0.001$) and N excretion was less for barrows fed the diet containing M422 than for those fed the diet containing M422-H.

Barrows fed the diet containing M422-H + iP retained more ($P = 0.05$) grams of P and excreted fewer ($P = 0.05$) grams of urinary Ca, and barrows absorbed and retained less ($P \leq 0.02$) N (g/d and % of intake) than those fed the diets containing M955, NB + iP, M422 + iP, or M635 + iP (diet 9 vs. diets 4 + 5 + 6 + 7, all with 0.30% aP). Even though barrows fed the diet containing M422-H + iP consumed, absorbed, and retained fewer ($P = 0.01$) kilocalories of energy per day than barrows fed the other diets with 0.30% aP, the percentages of energy absorbed, retained, and excreted were not different for barrows fed these diets.

DISCUSSION

This experiment demonstrated that the linear reduction in phytic acid P (or linear increase in aP) in the diets containing the LPB cultivars without added iP resulted in linear increases in ADG, G:F, bone strength, bone fat-free dry weight, and the apparent absorption and retention of P and Ca (g/d and % of intake). Thacker et al. (2003, 2004) also found linear increases in the apparent digestibility (%) of P by finishing pigs fed diets containing LPB with decreasing concentrations of phytic acid. There were no linear increases in N and energy balance (g/d or %) or DM digestibility in the current experiment, which is in agreement with 2 LPB experiments with finishing pigs (Thacker et al., 2003, 2004) in which the digestibility coefficients of DM, N, and GE were not increased by decreasing the phytic acid concentration in LPB.

In the current experiment, barrows fed the diet containing M955 did not differ in growth performance, bone strength, or other bone measurements from barrows fed diets containing the near-isogenic cultivars NB, M422, or M635 with added iP to provide 0.30% aP, the same concentration of aP provided by the diet containing M955 without added iP. Diets with 0.30% aP met the NRC (1998) aP requirement for the barrows from wk 2 to 4 of this experiment. This indicates that the reduction in phytic acid did not have a negative effect on any other nutritional factors in these mutant LPB cultivars, which is in agreement with our previous experiment with young pigs (Veum et al., 2002) in which diets that contained LPB or NB were equal in nutritional value when both diets were equalized in aP by adding iP to the NB diet.

In the current experiment, barrows fed the diet containing M955 without added iP also had greater per-

Table 5. Effect of mutant barley cultivars on the apparent absorption, retention, and excretion of phosphorus, calcium, nitrogen, and energy, and DM digestibility[1]

	Diet number and description									
	1	2	3	4	5	6	7	8	9	
Item	NB, wild-type control	M422	M635	M955	NB + iP	M422 + iP	M635 + iP	Hull-less M422	Hull-less M422 + iP	SEM
ADFI,[2] g	1,184	1,231	1,304	1,248	1,382	1,433	1,326	1,220	1,277	47
Phosphorus										
Intake, g/d	4.86	4.82	5.24	5.10	6.88	7.15	6.26	5.08	6.78	0.21
Absorbed, g/d	2.59	2.99	3.31	3.54	4.06	4.87	4.15	2.97	4.41	0.15
Fecal, g/d	2.27	1.83	1.92	1.56	2.82	2.28	2.11	2.11	2.36	0.12
Urinary, g/d	0.10	0.16	0.17	0.17	0.30	0.21	0.14	0.19	0.15	0.06
Retained, g/d	2.49	2.83	3.14	3.37	3.77	4.66	4.02	2.78	4.26	0.14
Absorbed/intake, %	53.6	62.1	63.3	69.3	59.1	68.1	66.3	58.5	65.1	1.3
Retained/intake, %	51.4	58.7	60.1	66.0	55.1	65.2	64.1	54.6	62.8	1.4
Excreted[3]/intake, %	48.6	41.3	39.9	34.0	44.9	34.8	35.9	45.4	37.2	1.4
Calcium										
Intake, g/d	7.22	7.39	7.82	7.61	8.43	8.74	7.96	7.44	7.92	0.29
Absorbed, g/d	5.30	5.87	6.33	6.57	6.33	6.86	6.35	5.94	5.96	0.36
Fecal, g/d	1.92	1.52	1.49	1.04	2.10	1.88	1.61	1.50	1.96	0.24
Urinary, g/d	3.31	3.53	2.95	2.48	2.19	2.45	2.29	3.39	1.87	0.22
Retained, g/d	1.99	2.34	3.38	4.09	4.14	4.41	4.06	2.55	4.09	0.37
Absorbed/intake, %	73.4	79.4	80.9	86.3	75.1	78.5	79.8	79.8	75.3	3.0
Retained/intake, %	27.6	31.7	43.2	53.8	49.1	50.5	51.0	34.3	51.6	3.5
Excreted/intake, %	72.4	68.3	56.8	46.2	50.9	49.5	49.0	65.7	48.4	3.5
Nitrogen										
Intake, g/d	36.73	38.98	41.65	40.42	42.33	44.79	42.28	39.66	39.05	1.49
Absorbed, g/d	30.52	32.06	33.74	33.43	33.35	36.19	34.37	31.93	30.32	1.31
Fecal, g/d	6.21	6.92	7.91	6.99	8.98	8.60	7.91	7.73	8.73	0.55
Urinary, g/d	6.24	5.59	7.39	6.60	7.50	8.34	6.85	9.01	7.64	0.80
Retained, g/d	24.28	26.47	26.35	26.83	25.85	27.86	27.52	22.92	22.68	1.24
Absorbed/intake, %	83.1	82.2	81.0	82.7	78.8	80.9	81.3	80.5	77.6	1.2
Retained/intake, %	66.1	67.9	63.3	66.4	61.1	62.2	65.1	57.8	58.1	1.8
Excreted/intake, %	33.9	32.1	36.7	33.4	38.9	37.8	34.9	42.2	41.9	1.8
Energy										
Intake, Kcal GE/d	4,968	5,221	5,556	5,336	5,779	6,043	5,626	4,937	5,118	195
Absorbed, Kcal/d	4,171	4,433	4,646	4,547	4,759	5,082	4,638	4,145	4,256	168
Fecal, Kcal/d	797	788	910	789	1,020	961	988	792	862	54
Urinary, Kcal/d	56	62	94	65	51	81	80	57	74	14
Retained, Kcal/d	4,115	4,371	4,552	4,482	4,708	5,001	4,558	4,088	4,182	168
Absorbed/intake, %	84.0	84.9	83.6	85.2	82.3	84.1	82.4	84.0	83.2	0.8
Retained/intake, %	82.8	83.7	82.0	84.0	81.5	82.8	81.0	82.8	81.7	0.8
Excreted/intake, %	17.2	16.3	18.0	16.0	18.5	17.2	19.0	17.2	18.3	0.8
DM digestibility, %	84.8	85.9	84.7	86.2	83.4	85.4	83.7	86.0	85.1	0.8

[1]NB = normal barley; M = mutant; iP = inorganic P.
[2]Days 22 to 26, as-fed basis.
[3]Excreted = fecal + urinary.

centages of apparent P, N, and energy absorption and retention, and greater percentages of Ca and DM absorption than barrows fed diets containing the near-isogenic cultivars with added iP to provide 0.30% aP. These results indicate that the utilization of most nutrients is increased by decreasing the concentration of phytic acid in barley-based diets fed to young pigs. In an experiment with young pigs in which barley was the only source of dietary phytate (Veum et al., 2002), the absorption and retention of Ca (g/d and as a % of intake) and energy utilization (DE and ME as a % of intake) were greater for pigs fed diets containing LPB than for those fed NB diets.

In the current experiment, the energy utilization of barrows fed diets containing hulled or hull-less LPB did not differ, because the Mcal of ME/kg of diet was equalized for all diets by adjusting the concentration of added lard. However, in experiments with finishing pigs in which the dietary energy concentration was not equalized, the digestibility coefficients for energy were greater for pigs fed the diets containing hull-less barley compared with those fed hulled barley (Thacker et al., 2003, 2004).

A major nutritional or environmental result of feeding diets containing LPB with increasing aP was the significant reduction in P excretion, even when the diets

Table 6. Statistical significance for the apparent absorption, retention, and excretion of phosphorus, calcium, nitrogen, and energy, and DM digestibility[1]

	P-value				
Item	Linear	Quadratic	Diet 4 vs. diets 5 + 6 + 7	Diet 2 vs. diet 8	Diet 9 vs. diets 4 + 5 + 6 + 7
ADFI,[2] g	0.316	0.145	0.147	0.864	0.320
Phosphorus					
Intake, g/d	0.262	0.456	<0.001	0.390	0.081
Absorbed, g/d	<0.001	0.147	<0.001	0.926	0.129
Fecal, g/d	<0.001	0.643	<0.001	0.097	0.191
Urinary, g/d	0.500	0.587	0.770	0.731	0.457
Retained, g/d	<0.001	0.190	<0.001	0.797	0.052
Absorbed/intake, %	<0.001	0.072	<0.001	0.058	0.651
Retained/intake, %	<0.001	0.203	0.003	0.054	0.889
Excreted[3]/intake, %	<0.001	0.203	0.003	0.054	0.888
Calcium					
Intake, g/d	0.320	0.146	0.151	0.864	0.321
Absorbed, g/d	0.018	0.218	0.357	0.931	0.148
Fecal, g/d	0.016	0.960	0.003	0.942	0.290
Urinary, g/d	0.010	0.365	0.446	0.468	0.052
Retained, g/d	<0.001	0.590	0.590	0.652	0.720
Absorbed/intake, %	0.007	0.650	0.008	0.933	0.157
Retained/intake, %	<0.001	0.750	0.653	0.631	0.681
Excreted/intake, %	<0.001	0.750	0.652	0.631	0.681
Nitrogen					
Intake, g/d	0.300	0.320	0.542	0.221	0.037
Absorbed, g/d	0.363	0.657	0.724	0.456	0.007
Fecal, g/d	0.495	0.095	0.016	0.120	0.348
Urinary, g/d	0.345	0.800	0.410	<0.001	0.767
Retained, g/d	0.720	0.750	0.368	0.068	0.003
Absorbed/intake, %	0.800	0.150	0.014	0.298	0.015
Retained/intake, %	0.472	0.421	0.019	<0.001	0.010
Excreted/intake, %	0.472	0.422	0.019	<0.001	0.010
Energy					
Intake, Kcal GE/d	0.180	0.104	0.453	0.312	0.012
Absorbed, Kcal/d	0.136	0.157	0.983	0.236	0.012
Fecal, Kcal/d	0.902	0.138	0.011	0.962	0.210
Urinary, Kcal/d	0.520	0.082	0.817	0.824	0.778
Retained, Kcal/d	0.148	0.203	0.998	0.243	0.012
Absorbed/intake (DE), %	0.444	0.528	0.010	0.481	0.707
Retained/intake, %	0.480	0.245	0.005	0.439	0.508
Excreted/intake, %	0.480	0.245	0.005	0.439	0.508
DM digestibility, %	0.380	0.581	0.024	0.887	0.602

[1]The linear and quadratic contrasts included diets 1 to 4 that contained normal barley (NB), Mutant (M) 422, M635, or M955, respectively, without added inorganic P (iP). Diet 4 provided 0.30% available P (aP) without added iP. Diets 5, 6, and 7 contained NB, M422, or M635, respectively, with added iP to provide 0.30% aP. Diet 8 contained hull-less M422 without added iP. Diet 9 contained hull-less M422 with added iP to provide 0.30% aP.
[2]Days 22 to 26, as-fed basis.
[3]Excreted = fecal + urinary.

containing M422 or M635 were supplemented with iP to meet the aP requirement. Fecal plus urinary excretion of P in grams per day was reduced by 44.6, 27.9, and 20.2% in barrows fed diets containing M955, M635 + iP, or M422 + iP, respectively, compared with barrows fed the diet containing NB + iP when iP was added to equalize aP at 0.30%. This indicates that the reduction in P excretion is directly proportional to the reduction in phytic acid in the mutant barley. However, all the barley mutants were effective in reducing P excretion, even when small amounts of added iP were required in the diets containing M422 or M635 to provide 0.30% aP. Therefore, selecting the LPB with the most desirable agronomic characteristics for commercial development will greatly reduce P excretion regardless of the mutant cultivar chosen. Other experiments have also found greater apparent digestibility coefficients (%) for finishing pigs fed LPB compared with finishing pigs fed NB (Thacker et al., 2003, 2004).

In another experiment in which barley mutants were evaluated in diets fed to young pigs, P excretion (g/d, feces plus urine) was reduced by 16 and 55%, respectively, by LPB in fortified barley-SBM or semipurified diets compared with NB (Veum et al., 2002). The higher

apparent digestibility coefficients for pigs fed the diets containing LPB in the current experiment are within the range of values reported for young pigs (Veum et al., 2002) and finishing pigs (Thacker et al., 2003). Replacing NB with LPB in poultry diets also increased P availability (Li et al., 2001b) and reduced P excretion in poultry waste (Li et al., 2001a). Low-phytate corn was also effective in increasing P availability and reducing P excretion compared with normal corn in diets fed to swine (Spencer et al., 2000; Veum et al., 2001; Xavier et al., 2004) and poultry (Li et al., 2000).

The future development of low-phytate grains (Rasmussen and Hatzack, 1998; Raboy et al., 2001; Dorsch et al., 2003) and low-phytate soybeans (Wilcox et al., 2000; Hitz et al., 2002; Oltmans et al., 2005) has the potential to greatly increase P utilization and reduce P excretion in diets fed to young poultry and swine. Low-phytate grains and soybeans will have a major impact on improving nonruminant animal and human nutrition globally because the genetically transmitted low-phytate trait is present in the grain at harvest. Therefore, no dietary supplements or grain processing is required to utilize this nutritional benefit, plus they have the added environmental benefit of greatly reducing P pollution (Raboy, 2001, Raboy et al., 2001). Diets containing low-phytate grains and low-phytate oilseed meals may also be pelleted without any negative effect on the low-phytate trait. However, plant breeding requires a long-term effort that may be delayed by many potential genetic and agronomic problems, such as maintaining acceptable crop yields and the need to breed the low-phytate trait into other phytate-containing plant feed ingredients.

Supplementation of grain-oilseed meal diets with a commercially produced phytase enzyme product is an established and practical approach available to increase P utilization and reduce P excretion in diets fed to growing swine (Liu et al., 1997, 1998; Veum et al., 2006) and poultry (Ledoux et al., 1995; Augspurger and Baker, 2004; Onyango et al., 2005). Practical limitations of using a phytase product are the losses in phytase enzyme activity that occur when the diets are pelleted because of the heat generated, and the gradual loss of phytase activity that occurs during diet storage, with the loss of enzyme activity dependent upon the stability characteristics of the specific phytase product used (Jongbloed and Kemme, 1990; Coelho, 1996a; Silversides et al., 2004). To prevent the losses of enzyme activity that occur during pelleting, a liquid phytase enzyme product may be sprayed on the pellets immediately after pelleting during the cooling and drying procedure (Coelho, 1996b; Kapp and McKnight, 1996). The use of a phytase enzyme product in combination with a low-phytate grain may also have nutritional and environmental applications in specialized feeding situations (Xavier et al., 2003, 2004).

In conclusion, the apparent utilization of P and Ca; bone strength, bone fat-free dry weight and ash weight; and growth performance increased with increasing dietary concentrations of aP provided by low-phytic acid barley, with linear decreases in P and Ca excretion. Pigs fed the diet containing M955, the mutant with the highest percentage of aP, had greater utilization and less excretion of P, Ca, N, energy, and DM than pigs fed diets containing the near-isogenic NB, M422, or M635 cultivars with supplemental iP to equalize aP at 0.30%. There were no differences in energy utilization by pigs fed diets with hulled or hull-less LPB when the ME/kg of diet was equalized by adjusting the addition of lard.

Commercial development of low-phytic acid barley cultivars for feeding swine will increase the utilization and decrease the excretion of phosphorus and calcium in swine waste. The diet containing the low-phytic acid mutant barley with the lowest concentration of phytic acid met the available phosphorus requirement of the barrows in this experiment without supplemental inorganic phosphorus, and the barrows fed that diet had greater utilization and less excretion of phosphorus, calcium, nitrogen, energy, and DM than barrows fed one of the other diets containing a near-isogenic low-phytic acid mutant or normal barley supplemented with inorganic phosphorus to provide the same concentration of available phosphorus. Compared with normal barley, diets containing the low-phytic acid mutant barley cultivars reduced phosphorus excretion by 20.2 to 44.6%. This environmental benefit will contribute to the sustainability of swine production worldwide.

LITERATURE CITED

Angel, C. R., W. J. Powers, T. J. Applegate, N. M. Tamim, and M. C. Christman. 2005. Influence of phytase on water-soluble phosphorus in poultry and swine manure. J. Environ. Qual. 34:563–571.

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Offic. Anal. Chem., Arlington, VA.

Augspurger, N. R., and D. H. Baker. 2004. High dietary phytase levels maximize phytate-phosphorus utilization but do not affect protein utilization in chicks fed phosphorus- or amino acid-deficient diets. J. Anim. Sci. 82:1100–1107.

Benson, J. V., Jr., and J. A. Patterson. 1971. Chromatographic advances in amino acid and peptide analysis using spherical resins and their applications in biochemistry and medicine. Pages 1–73 in New Techniques in Amino Acid, Peptide and Protein Analysis. A. Niederweiser and G. Pataki, ed. Ann Arbor Sci. Publ., Ann Arbor, MI.

Coelho, M. B. 1996a. Practical phytase stability. Pages 667–671 in Phytase in Animal Nutrition and Waste Management. BASF Reference Manual DC9601. M. B. Coelho and E. T. Kornegay, ed. BASF Corporation, Mount Olive, NJ.

Coelho, M. B. 1996b. Selection of product concentration and form (dry or liquid). Pages 673–679 in Phytase in Animal Nutrition and Waste Management. BASF Reference Manual DC9601. M. B. Coelho and E. T. Kornegay, ed. BASF Corporation, Mount Olive, NJ.

Crenshaw, T. D. 2001. Calcium, phosphorus, vitamin D, and vitamin K in swine nutrition. Pages 187–212 in Swine Nutrition. A. J. Lewis and L. L. Southern, ed. CRC Press, Boca Raton, FL.

Crenshaw, T. D. 1986. Reliability of dietary Ca and P levels and bone mineral content as predictors of bone mechanical properties at various time periods in growing swine. J. Nutr. 116:2155–2170.

Cromwell, G. L., and R. D. Coffey. 1991. Phosphorus—A key essential nutrient, yet a possible major pollutant—Its central role in animal nutrition. Pages 133–145 in Biotechnology in the Feed Industry, Proc. Alltech's 7th Annu. Symp. T. P. Lyons, ed. Alltech Technical Publications, Nicholasville, KY.

Cromwell, G. L., M. D. Lindemann, G. R. Parker, R. D. Coffey, H. J. Monegue, and J. H. Randolph. 1995. Effects of dietary calcium on the efficacy of phytase and on the utilization of phosphorus in low phosphorus diets for pigs. J. Anim. Sci. 73(Suppl. 1):73. (Abstr.)

Dorsch, J. A., A. Cook, K. A. Young, J. M. Anderson, A. T. Bauman, C. J. Volkmann, P. P. N. Murthy, and V. Raboy. 2003. Seed phosphorus and inositol phosphate phenotype of barley *low phytic acid* genotypes. Phytochemistry 62:691–706.

Harper, A. F., E. T. Kornegay, and T. C. Schell. 1997. Phytase supplementation of low-phosphorus growing-finishing pig diets improves performance, phosphorus digestibility, and bone mineralization and reduces phosphorus excretion. J. Anim. Sci. 75:3174–3186.

Hitz, W. D., T. J. Carlson, P. S. Kerr, and S. A. Sebastian. 2002. Biochemical and molecular characterization of a mutation that confers a decreased raffinosaccharide and phytic acid phenotype on soybean seeds. Plant Physiol. 128:650–660.

Johnston, S. L., S. B. Williams, L. L. Southern, T. D. Bidner, L. D. Bunting, J. O. Matthews, and B. M. Olcott. 2004. Effect of phytase addition and dietary calcium and phosphorus levels on plasma metabolites and ileal and total-tract nutrient digestibility in pigs. J. Anim. Sci. 82:705–714.

Jongbloed, A. W., and P. A. Kemme. 1990. Effect of pelleting mixed feeds on phytase activity and the apparent absorbability of phosphorus and calcium in pigs. Anim. Feed Sci. Technol. 28:233–242.

Kapp, A., and W. F. McKnight. 1996. Liquid application systems for Natuphos® phytase. Pages 687–692 in Phytase in Animal Nutrition and Waste Management. BASF Reference Manual DC9601. M. B. Coelho and E. T. Kornegay, ed. BASF Corporation, Mount Olive, NJ.

Larson, S. R., K. A. Young, A. Cook, T. K. Blake, and V. Raboy. 1998. Linkage mapping of two mutations that reduce phytic acid content of barley grain. Theor. Appl. Genet. 97:141–146.

Ledoux, D. R., K. Zyla, and T. L. Veum. 1995. Substitution of phytase for inorganic phosphorus for turkey hens. J. Appl. Poult. Res. 4:157–163.

Lei, X. G., P. K. Ku, E. R. Miller, M. T. Yokoyama, and D. E. Ullrey. 1994. Calcium level affects the efficacy of supplemental microbial phytase in corn-soybean meal diets of weanling pigs. J. Anim. Sci. 72:139–143.

Leytem, A. B., B. L. Turner, and P. A. Thacker. 2004. Phosphorus composition of manure from swine fed low-phytate grains: Evidence for hydrolysis in the animal. J. Environ. Qual. 33:2380–2383.

Li, Y. C., D. R. Ledoux, T. L. Veum, V. Raboy, and D. S. Ertl. 2000. Effects of low-phytic acid corn on phosphorus utilization, performance, and bone mineralization in broiler chicks. Poult. Sci. 79:1444–1450.

Li, Y. C., D. R. Ledoux, T. L. Veum, V. Raboy, and K. Zyla. 2001a. Low-phytic acid barley Improves performance, bone mineralization, and phosphorus retention in turkey poults. J. Appl. Poult. Res. 10:178–185.

Li, Y. C., D. R. Ledoux, T. L. Veum, V. Raboy, K. Zyla, and A. Wikiera. 2001b. Bioavailability of phosphorus in low-phytic acid barley. J. Appl. Poult. Res. 10:86–91.

Liu, J., D. W. Bollinger, D. R. Ledoux, M. R. Ellersieck, and T. L. Veum. 1997. Soaking increases the efficacy of supplemental microbial phytase in a low-phosphorus corn-soybean meal diet for growing pigs. J. Anim. Sci. 75:1292–1298.

Liu, J., D. W. Bollinger, D. R. Ledoux, and T. L. Veum. 2000. Effects of dietary calcium: Phosphorus ratios on apparent absorption of calcium and phosphorus in the small intestine, cecum, and colon of pigs. J. Anim. Sci. 78:106–109.

Liu, J., D. W. Bollinger, D. R. Ledoux, and T. L. Veum. 1998. Lowering the dietary calcium to total phosphorus ratio increases phosphorus utilization in low-phosphorus corn-soybean meal diets supplemented with microbial phytase for growing-finishing pigs. J. Anim. Sci. 76:808–813.

Lott, J. N. A., I. Ockenden, V. Raboy, and G. D. Batten. 2000. Phytic acid and phosphorus in crop seeds and fruits: A global estimate. Seed Sci. Res. 10:11–33.

Maga, J. A. 1982. Phytate: Its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. J. Agric. Food Chem. 30:1–9.

Nielsen, N. C., S. Andersen, A. Madsen, and H. P. Mortensen. 1971. Dietary calcium-phosphorus ratios for growing pigs in relation to serum levels and bone development. Acta Vet. Scand. 12:202–219.

NRC. 1998. Nutrient Requirements of Swine. 10th rev. ed. Natl. Acad. Press, Washington, DC.

Oltmans, S. E., W. R. Fehr, G. A. Welke, V. Raboy, and K. Peterson. 2005. Agronomic and seed traits of soybean lines with low-phytate phosphorus. Crop Sci. 45:593–598.

Onyango, E. M., M. R. Bedford, and O. Adeola. 2005. Efficacy of an evolved *Escherichia coli* phytase in diets of broiler chicks. Poult. Sci. 84:248–255.

Partridge, I. G. 1978. Studies on digestion and absorption in the intestines of growing pigs 3. Net movements of mineral nutrients in the digestive tract. Br. J. Nutr. 39:527–537.

Pointillart, A., N. Fontaine, and M. Thomasset. 1984. Phytate phosphorus utilization and intestinal phytases in pigs fed low phosphorus: Wheat or corn diets. Nutr. Rep. Int. 29:473–483.

Pointillart, A., A. Fourdin, and N. Fontaine. 1987. Importance of cereal phytase activity for phytate phosphorus utilization by growing pigs fed diets containing triticale or corn. J. Nutr. 117:907–913.

Qian, H., E. T. Kornegay, and D. E. Conner, Jr. 1996. Adverse effects of wide calcium:phosphorus ratios on supplemental phytase efficacy for weanling pigs fed two dietary phosphorus levels. J. Anim. Sci. 74:1288–1297.

Raboy, V. 2001. Seeds for a better future: 'Low phytate' grains help to overcome malnutrition and reduce pollution. Trends Plant Sci. 6:458–462.

Raboy, V., P. E. Gerbasi, K. A. Young, S. D. Stoneberg, S. G. Pickett, A. T. Bauman, P. P. N. Murthy, W. F. Sheridan, and D. S. Ertl. 2000. Origin and seed phenotype of maize low phytic acid 1-1 and low phytic acid 2-1. Plant Physiol. 124:355–368.

Raboy, V., K. A. Young, J. A. Dorsch, and A. Cook. 2001. Genetics and breeding of seed phosphorus and phytic acid. J. Plant Physiol. 158:489–497.

Rasmussen, S. K., and F. Hatzack. 1998. Identification of two low-phytate barley (*Hordeum vulgare* L.) grain mutants by TLC and genetic analysis. Hereditas 129:107–112.

Reddy, N. R., M. D. Pierson, S. K. Sathe, and D. K. Salunkhe. 1989. Phytates in Cereals and Legumes. CRC Press, Boca Raton, FL.

Roslinsky, V., P. B. Eckstein, V. Raboy, B. G. Rossnagel, and G. L. Scoles. 2007. Molecular marker development and linkage analysis in three low phytic acid barley (*Hordeum vulgare*) mutant lines. Mol. Breed.

Silversides, F. G., T. A. Scott, and M. R. Bedford. 2004. The effect of phytase enzyme and level on nutrient extraction by broilers. Poult. Sci. 83:985–989.

Snedecor, G. W., and W. G. Cochran. 1989. Statistical Methods. 8th ed. Iowa State University Press, Ames, IA.

Spencer, J. D., G. L. Allee, and T. E. Sauber. 2000. Phosphorus bioavailability and digestibility of normal and genetically modified low-phytate corn for pigs. J. Anim. Sci. 78:675–681.

Spies, J. R., and D. C. Chambers. 1949. Chemical determination of tryptophan in proteins. Procedure N. Anal. Chem. 21:1249–1266.

Thacker, P. A., B. G. Rossnagel, and V. Raboy. 2004. Effect of phytase supplementation on phosphorus digestibility in low-phytate barley fed to finishing pigs. Arch. Anim. Nutr. 58:61–68.

Thacker, P. A., B. G. Rossnagel, and V. Raboy. 2003. Phosphorus digestibility in low-phytate barley fed to finishing pigs. Can. J. Anim. Sci. 83:101–104.

US Grains Council. 2006. About the Council: 2005 Annual Report. Barley, Corn and Sorghum. Available: http://www.grains.org/index.ww Accessed June 20, 2006. Washington, DC, 2005.

Veum, T. L., D. W. Bollinger, C. E. Buff, and M. R. Bedford. 2006. A genetically engineered *Escherichia coli* phytase improves nutrient utilization, growth performance, and bone strength of young swine fed diets deficient in available phosphorus. J. Anim. Sci. 84:1147–1156.

Veum, T. L., D. R. Ledoux, D. W. Bollinger, V. Raboy, and A. Cook. 2002. Low-phytic acid barley improves calcium and phosphorus utilization and growth performance in growing pigs. J. Anim. Sci. 80:2663–2670.

Veum, T. L., D. R. Ledoux, V. Raboy, and D. S. Ertl. 2001. Low-phytic acid corn improves nutrient utilization for growing pigs. J. Anim. Sci. 79:2873–2880.

Wilcox, J. R., G. S. Premachandra, K. A. Young, and V. Raboy. 2000. Isolation of high seed inorganic P, low-phytate soybean mutants. Crop Sci. 40:1601–1605.

Xavier, E. G., G. L. Cromwell, and M. D. Lindemann. 2003. Phytase additions to conventional or low-phytate corn-soybean meal diets on performance, bone traits, and phosphorus excretion of growing pigs. J. Anim. Sci. 81(Suppl. 1):100. (Abstr.)

Xavier, E. G., L. A. Pettey, G. L. Cromwell, and M. D. Lindemann. 2004. Phosphorus excretion of pigs fed conventional or low-phytate corn-soybean meal diets without or with phytase. J. Anim. Sci. 82(Suppl.1):253–254. (Abstr.)

Zyla, K., M. Czubak, J. Roznowski, and T. Fortuna. 2002. Changes in the activity of endogenous phytase in germinating sesame seeds. Pages 137–138 in Proc. 33rd Scientific Meeting Food Chem. and Technol. Comm., Lublin, Poland. Polish Acad. of Sci., Lublin, Poland.